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L1: Entry 24 of 32 File: USPT Sep 8, 1998

DOCUMENT-IDENTIFIER: US 5804382 A
TITLE: Methods for identifying differentially expressed genes and differences between genomic nucleic acid sequences

ABPL:
Methods for identifying differentially expresses genes and differences between genomic nucleic acid sequences are described. These methods typically include: (a) providing a tester DNA molecule with an amplification tag at the 5' and 3' ends of the molecule and a driver DNA or RNA molecule lacking said amplification tag; (b) hybridizing said tester and said driver molecules to form a reaction mixture, wherein said reaction mixture comprises a tester--tester homoduplex, a tester-driver heteroduplex, a driver--driver homoduplex, a single stranded driver DNA molecule and a single stranded tester DNA molecule; (c) treating said reaction mixture to reduce the number of single stranded molecules in said mixture; (d) treating said reaction mixture to remove said amplification tag from said tester-driver heteroduplex; and (e) amplifying said tester--tester homoduplex from said reaction mixture to form an amplification product, wherein steps (c) and (d) occur before step (e).

BSPR:
Yet another aspect of the invention pertains to a method for identifying a difference between genomic DNA sequences. The method includes (a) providing a tester DNA molecule with an amplification tag at the 5' and 3' ends of the molecule and a driver DNA molecule lacking the amplification tag, (b) hybridizing the tester and the driver molecules to form a reaction mixture, wherein the reaction mixture comprises a tester--tester homoduplex, a tester-driver heteroduplex, a driver--driver homoduplex, a single-stranded driver DNA molecule and a single-stranded tester DNA molecule, (c) treating the reaction mixture to reduce the number of single-stranded molecules in the mixture and to remove the amplification tag from the tester-driver heteroduplex, and (d) amplifying the tester--tester homoduplex from the reaction mixture to form an amplification product, wherein step (c) occurs before step (d).

DEPR:
The present invention features a methods for identifying a differentially expressed gene or differences in genomic nucleic acid sequences. The method includes (a) providing a tester DNA molecule with an amplification tag at the 5' and 3' ends of the molecule and a driver DNA or RNA molecule lacking the amplification tag, (b) hybridizing the tester and the driver molecules to form a reaction mixture, wherein the reaction mixture comprises a tester--tester homoduplex, a tester-driver heteroduplex, a driver--driver homoduplex, a single-stranded driver DNA molecule and a single stranded tester DNA molecule, (c) treating the reaction mixture to reduce the number of single-stranded molecules in the mixture, (d) treating the reaction mixture to remove the amplification tag from the tester-driver heteroduplex, and (e) amplifying the tester--tester homoduplex from the reaction mixture to form an amplification product, wherein steps (c) and (d) occur before step (e). In one embodiment, steps (c) and (d) occur simultaneously. In another embodiment, steps (b) to (e) are repeated at least once and preferably at least three times.

DEPR:
The terms "tester DNA molecule" and "driver DNA molecule" refer to nucleic

acid molecules, e.g., deoxyribonucleic acid molecules, single-stranded DNA molecules, double-stranded cDNA molecules, or genomic DNA molecules. Tester and driver DNA molecules are typically isolated from different sources, e.g., two different types of cells, such as a normal cell and a cancer cell, a highly malignant cell and a low malignant cell, the same cell type from two different organisms, e.g., different organisms within the same species or different organisms from different species, or two cells incubated or exposed to different conditions, e.g., incubated in vitro in the presence or absence of one or more of the following of, for example, cytokines, growth factors, or other biologically active molecules, or exposed to different environmental or stress conditions, e.g., UV, heat, or chemicals such as drugs. The term "driver RNA molecule" refers to a nucleic acid molecule, e.g., a ribonucleic acid molecule, preferably, an mRNA molecule. The driver RNA molecule can be isolated from the sources described herein for the tester and driver DNA molecules.

DEPR:
This method is designed to isolate genes expressed differentially between two cell types or between cells treated in two different ways or for isolation of differences between genomic DNA sequences (FIGURE 1). In the first step, both tester DNA and driver DNA are prepared. This is accomplished by digesting the double-stranded cDNA with restriction enzymes of choice, ligating the fragments to amplification tags, e.g., linkers, and carrying out a PCR reaction with linker sequence as primer. The driver DNA is digested with restriction enzymes to remove the linker sequence. In the second step, the tagged/linkered tester DNA is hybridized to an excess of driver DNA (with tags/linkers removed) followed by incubation with mung bean nuclease which digests single-stranded DNA specifically. This leaves only linkered tester--tester homohybrids and unlinkered homo- and heteroduplexes. In the following step, the linkered tester--tester homoduplexes are amplified by PCR with tag/linker sequence as primer to fulfill the first round of enrichment. The amplified PCR products are then used as tester for another round of subtraction. The process of subtractive hybridization, mung bean nuclease digestion, and PCR amplification is carried out three times. Finally, the PCR products of the third round of subtraction are used to prepare a subtraction library by inserting them into a vector.

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L2: Entry 1 of 2 File: USPT Jun 2, 1998

DOCUMENT-IDENTIFIER: US 5759822 A
TITLE: Method for suppressing DNA fragment amplification during PCR

DEPR:
In another embodiment, the subject invention can be used to subtract a cDNA library (tester) against a set of any cDNAs that have already been cloned into any particular vector (driver). This application of the subject invention is particularly useful for cDNA sequencing projects, such as the human genome sequencing project, where a random strategy to clone all individual cDNAs which are present in a cDNA library has been used. Subtraction of already sequenced cDNAs from a cDNA library should increase the efficiency of identification and sequencing of novel cDNAs which are absent from cDNA databases. Also, the subject invention can be used with subtractive hybridization for identifying differences between tester and driver genomic DNAs. This application of the subject invention is similar to subtractive hybridization of cDNAs, but can be used for discovering probes for pathogenic organisms, for identifying otherwise anonymous loci that have suffered genetic rearrangements, and for detecting the polymorphisms located near the genes affected by inherited disorders.

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